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PMRA Sub. No. 1999-1169/TOA  
Iprovalicarb/IVB

~ PROTECTED ~

Sub-chronic (90-d) Oral Toxicity / 1  
DACO 4.3.1 / OECD IIA 5.3.2Reviewer: S. Semalulu, Date April 17, 2001

**STUDY TYPE:** Sub-chronic Oral Toxicity [feeding-rat]; OPPTS 870.3100 (rodent), [§82-1]; OECD 408.

**TEST MATERIAL (PURITY):** SZX 0722 (99.4%) [Iprovalicarb]

**SYNONYMS:** Melody

**CITATION:** Schladt, L., Wattag-Gebert, B. and M. Rinke (1996): SZX 0722 - Sub-chronic Toxicological investigations in Wistar Rats (Administration in feed over 13 weeks followed by a 4-week recovery period.) Bayer AG, Wuppertal, Germany. Report no. 24766 (February 7, 1996); Unpublished

**SPONSOR:** Bayer Corporation.

**EXECUTIVE SUMMARY:**

In a sub-chronic toxicity study (MRID not available), SZX 0722 technical (98.1 - 98.7 %) was administered to Wistar rats (10/sex/group), in the diet at dose levels of 0, 1250, 5000, 20000 ppm (0, 87.4/133.9, 372.7/561.4, 1524.0/2585 mg/kg bw/day, males/females), for 13 weeks. Recovery groups of 10 rats/sex were treated at levels of 0 or 20000 ppm for 13 weeks, and observed for an additional four weeks after termination of treatment, for evidence of recovery. There were no treatment-related clinical signs, nor mortalities in any dose group. At 20000 ppm, there was increased food intake (17 %) and decreased food efficiency (food intake increased (23 %) relative to body weight gained) among females, which persisted during the recovery period. There was also a slight reduction in body weight gain in both sexes (males: 6 %, females: 9 %, compared to controls) which persisted throughout the recovery periods. In addition, there was a slight increase in leukocyte counts in males, but was absent following the recovery period. Terminal plasma cholesterol levels were increased in females, alkaline phosphatase activity was slightly elevated, and plasma triglycerides were decreased in males, at 20000 ppm. Six of 8 males of the 20000 ppm dose group had pale livers. Absolute liver weights were increased in females at 20000 ppm but relative liver weights were increased in both sexes at that dose. There were no treatment-related histopathological changes in any of the tissues examined. Liver N-demethylase activity was increased in females at 20000 ppm whereas O-demethylase activity was increased in both sexes at 5000 ppm and above. Liver cytochrome P-450 levels were elevated in males at 20000 ppm and in females at 1250 ppm and above. These liver microsomal enzyme changes were interpreted as indicators of enzyme induction, and not necessarily as indicators of overt toxicity.

The LOAEL for both sexes was 20000 ppm (1524.0 or 2585.9 mg/kg bw/d in males and females respectively), based on treatment related increased food intake with decrease in body weight gain and food efficiency, and increased plasma cholesterol levels in females, decrease in plasma triglycerides and increases in leukocyte counts, alkaline phosphatase levels and incidence of pale livers in males, as well as increased relative liver weights, in both sexes. The NOAEL was 5000 ppm (372.7/561.4 mg/kg bw/day in males/females).

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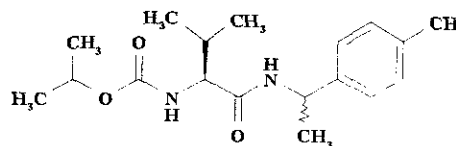
This sub-chronic toxicity study is classified acceptable and satisfies the guideline requirement for a sub-chronic oral study (OPPTS 870.3100; OECD 408) in the rat.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

## I. MATERIALS AND METHODS

### A. MATERIALS:

- 1 **Test Material:** SZX 0722  
**Description:** Technical, a white powder  
**Lot/Batch #:** NLL 4812-6.1  
**Purity:** 99.4 % a.i.  
**Compound Stability:** Stable at room temperature  
**CAS #:**  
 Structure



- 2 **Vehicle and/or positive control:** 1 % DAB 9 Peanut (Batch # 904) to minimize dust formation.

- 3 **Test animals:**  
**Species:** Rat  
**Strain:** Bor:WISW (SPF-Cpb)  
**Age/weight at study initiation:** 5-6 weeks old /140-180 males; 108-136 g females.  
**Source:** Winkelmann Experimental Animal Breeders, Brochen, Kreis Paderborn  
**Housing:** Group caged by sex in Type III macrolon during acclimatization, and individually caged in Type II Macrolon cages during dosing.  
**Diet:** Altromin rat maintenance diet, # 1324 (during acclimatization), and # 1321 during dosing (Altromin, GmbH, Lage), fed *ad libitum*  
**Water:** Tap water in polycarbonate bottles, provided *ad libitum*  
**Environmental conditions:**  
**Temperature:**  $22 \pm 2^\circ \text{C}$   
**Humidity:**  $55 \pm 5\%$   
**Air changes:** 15-20/hr  
**Photoperiod:** 12 hrs dark/ 12 hrs artificial light  
**Acclimation period:** 7 days

### B. STUDY DESIGN:

1. **In life dates** - Start: March 1993 - End: July 1993.

**2. Animal assignment:**

Animals were assigned randomly to the test groups shown in Table 1, using a computer generated list of random numbers. The doses were based on findings from a subacute toxicity study in rats, which indicated treatment related effects on body weight development, food intake and alkaline phosphatase (males only) at 20000 ppm, and on plasma cholesterol, plasma triglycerides and absolute liver weights in females at 6000 ppm, and on plasma creatinine and urea concentration in males as well as induction of liver microsomal enzymes (cytochrome P-450, and O-demethylase) in both sexes at 6000 ppm and above.

**TABLE 1: Study design**

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg bw)		# Male	# Female
		male	female		
Control	0	0	0	10	10
Low	1250	87.4	133.9	10	10
Mid	5000	372.7	561.4	10	10
High	20000	1524	2585	10	10
Recovery Control	0	0	0	10	10
Recovery High	20000	1524	2585	10	10

**3. Diet preparation and analysis**

Diet was prepared weekly by mixing appropriate amounts of test substance with the maintenance food and was stored at room temperature. Homogeneity of the diet mixture and stability were tested on feed samples collected. During the study, treated food samples were collected three times and analysed for the concentration of the test material.

**Results - Homogeneity Analysis:** 91.0 - 101 % of nominal concentration

**Stability Analysis:** 101 - 108% of nominal concentration over a 14 day period

**Concentration Analysis:** 97.0 - 103% of nominal concentration

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

**4. Statistics** - Arithmetic group means and standard deviations were calculated from the individual animal results for body weight, food and water intake, organ weights, and hematological and clinical chemistry parameters. Other test results were compared between cohorts and controls using the rank tests (U tests) of Mann and Whitney, and Wilcoxon. Significant levels were set at  $\alpha = 5\%$  ( $p \leq 0.05$ ) and  $\alpha = 1\%$  ( $p \leq 0.01$ ). The statistical methods used are acceptable.

**C. METHODS:****1. Observations:**

Animals were inspected at least once daily for signs of toxicity and mortality.

**2. Body weight:**

Animals were weighed prior to study initiation, at the beginning of each study week and immediately prior to necropsy.

**3. Food consumption and compound intake:**

Food and water intakes for each individual animal were determined once weekly and at study termination. Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency [if given] (body weight gain in kg/food consumption in kg per unit time X 100) and compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

**4. Ophthalmoscopic examination:**

Eyes of all animals were examined prior to study initiation. The control and high dose group were subjected to further ophthalmological examination at study termination.

**5. Haematology and Clinical Chemistry:**

Blood was collected from five animals after an overnight fast for each dose group, at the end of the treatment period, for haematology and clinical analysis. For glucose analysis, blood was collected from either the distal vessels following tail resection or by puncture of retro-orbital sinus. Blood for other analyses was drawn by cardiac puncture under diethyl ether anaesthesia. To determine liver microsomal enzyme activities, liver sections (0.9 - 1.2 grams) were collected from each animal at necropsy.

The CHECKED (X) parameters in the table below were examined.

**a. Haematology**

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X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

\* Required for subchronic studies based on Subdivision F Guidelines

**b. Clinical Chemistry**

X	<b>ELECTROLYTES</b>	X	<b>OTHER</b>
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	<b>ENZYMES</b>	x	Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum protein (TP)*
	Cholinesterase (ChE)	x	Triglycerides
x	Creatine phosphokinase		Serum protein electrophores
x	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

\* Required for subchronic studies based on Subdivision F Guidelines

C. Clinical chemistry of liver tissues involved assays of N-demethylase, O-demethylase and cytochrome P-450 assays

**6. Urinalysis\***

Urine was collected from fasted animals at the end of the treatment period. The CHECKED (X) parameters were examined.

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X		X	
x	Appearance	x	Glucose
x	Volume	x	Ketones
x	Specific gravity	x	Bilirubin
x	pH	x	Blood
x	Sediment (microscopic)	x	Nitrate
x	Protein	x	Urobilinogen

\* Optional for sub-chronic studies

## 7. Sacrifice and Pathology

All animals that died, and those sacrificed on schedule were subjected to gross pathological examination and the tissues checked (x) were collected for histological examination. The organs marked (xx) were also weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta*	x	Brain*
x	Salivary glands*	x	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels) <sup>T</sup>
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen*	x	Eyes (optic n.) <sup>T</sup>
x	Jejunum*	x	Thymus*		
x	Ileum*				
x	Cecum*				
x	Colon*			x	<b>GLANDULAR</b>
x	Rectum*	x	<b>UROGENITAL</b>	x	Adrenal gland*
xx	Liver**	x	Kidneys*+	x	Lacrimal gland <sup>T</sup>
x	Gall bladder*	x	Urinary bladder*	x	Mammary gland <sup>T</sup>
x	Pancreas*	x	Testes**	x	Parathyroids***
		x	Epididymides	x	Thyroids***
		x	Prostate		
		x	Seminal vesicle		
	<b>RESPIRATORY</b>	x	Ovaries	x	<b>OTHER</b>
x	Trachea*	x	Uterus*	x	Bone
x	Lung*			x	Skeletal muscle
x	Nose			x	Skin
x	Pharynx			x	All gross lesions and masses*
x	Larynx				

\* Recommended for sub-chronic rodent studies based on Guideline 870.3100; + organ weights required for rodent studies;  
T= required only when toxicity or target organ.

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## II. RESULTS

### A. Observations :

#### 1. Clinical signs of toxicity

There were no treatment-related clinical signs in any of the test groups throughout the study.

#### 2. Mortality

One animal of either sex died in the 20000 ppm dose group during the study due to a blood sampling error. There was no mortality in any test groups throughout the study.

### B. Body weight and body weight gain:

The body weight gain is presented in Table 2 and Figs.1, 2,3). A slight decrease in body weight gain was observed at 20000 ppm in both sexes (max. 6 % males; 9 % females, compared to controls) and it persisted through the recovery period. The decrease in body weight gain was considered toxicologically significant in females only, because it was associated with significant decrease in food efficiency.

**Table 2. Body weight gain**

Dose (ppm)		0 (+ recovery)	1250	5000	20000	20000 + recovery
Body weight gain (g)	m	278 (316)	271	283	260	286
	f	112 (132)	109	103	92	115

Fig. 1: Mean body weights of male

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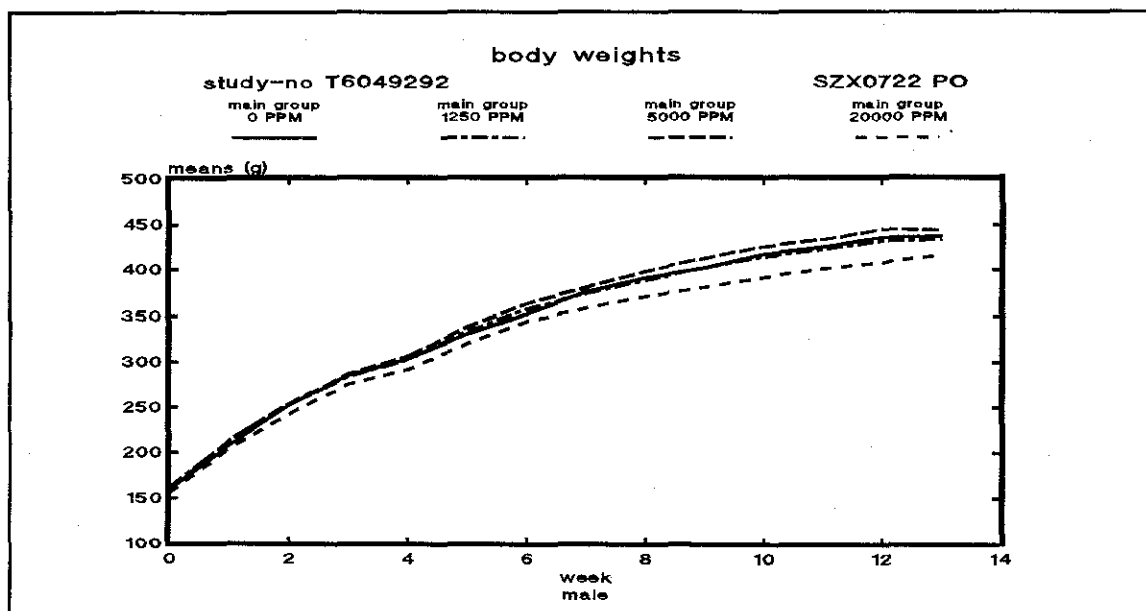
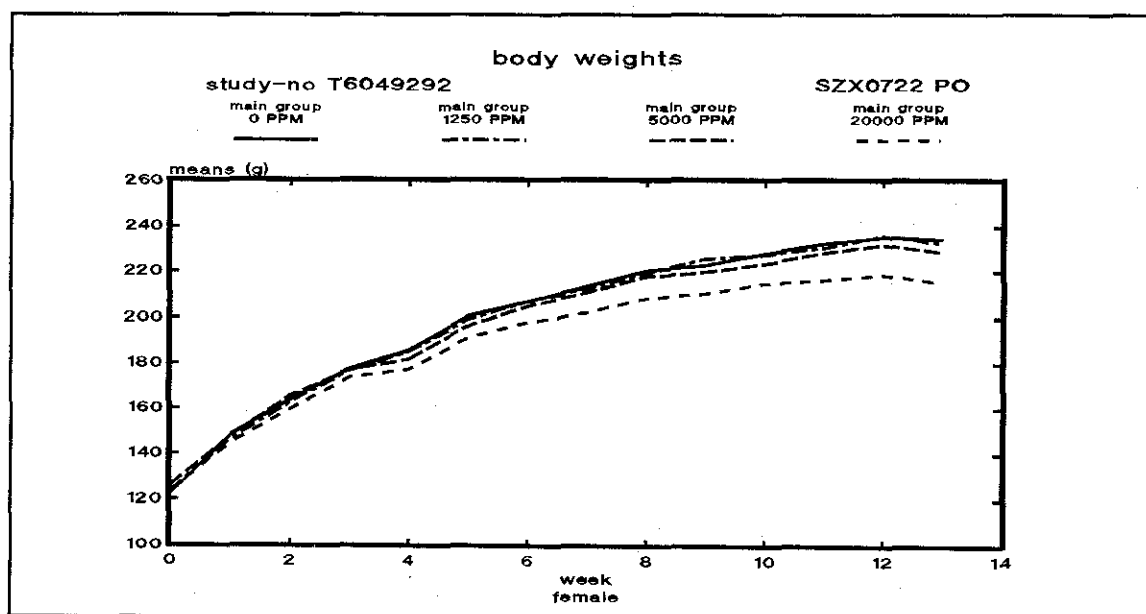


Fig.2 Mean body weights - females

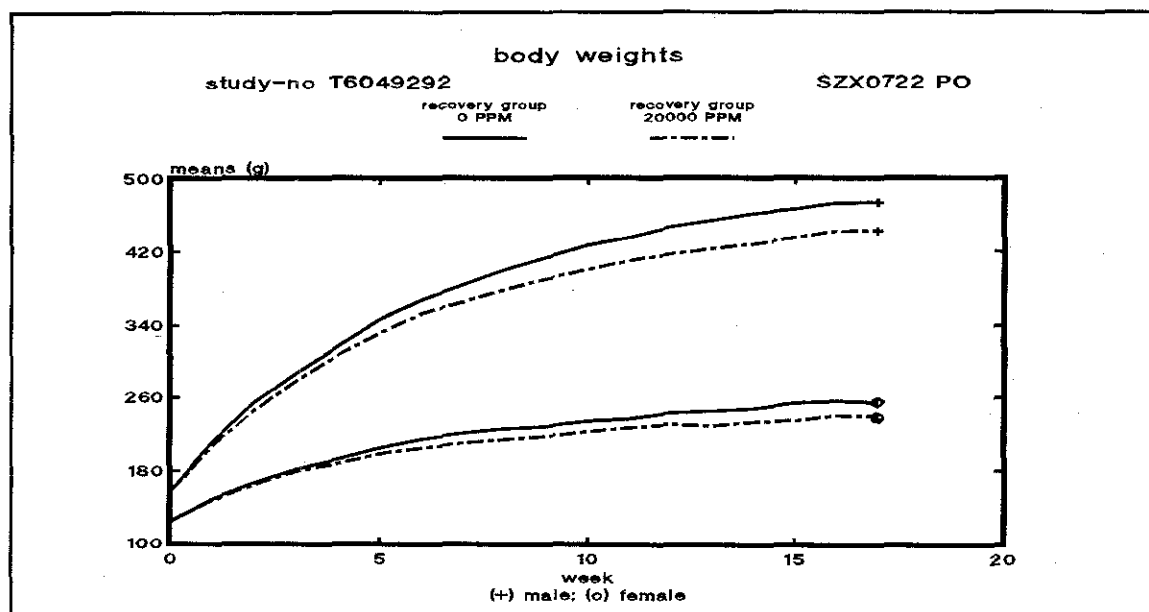


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Fig. 3 Mean body weights of recovery groups



**C. Food consumption and compound intake:****1. Food and water consumption**

Notable food consumption, and food to body weight conversion changes are presented in Table 3. At the end of the dosing period (day 92), there was an increase in food intake per animal (17 %), and food intake relative to the body weight gained (23 %) of females at 20000 ppm. At the end of the recovery period the average feed intake was still slightly higher than the control in females of that treatment-group. There was no treatment related effect on water consumption throughout the study.

Table 3. Mean food intake (g/animal/day) and food to body weight conversion [g/kg bw/d].

	0 ppm			1250 ppm	5000 ppm	20000 ppm		
<b>Females</b>	main groups	recovery groups		main groups	main groups	main groups	recovery groups	
<b>Day</b>	92	92	28	92	92	92	92	28
<b>[g/animal/d]</b>	21.1	22.0	19.3	21.5	22.3	24.6	26.1	20.8
<b>[g/kg bw/d]</b>	105.0	106.6	76.5	107.2	112.3	129.3	131.0	88.6

**2. Test compound consumption**

The calculated test compound intake (mg/kg bw/day) is presented in Table I. The test compound consumed by the test groups corresponded to the theoretical differences in the dosage factors used.

**3. Food efficiency**

There was a decrease in food efficiency in females at 20000 ppm, characterised by an increase (23%) in food intake relative to the body weight gained when compared to controls. This was considered a toxicologically significant treatment-related effect.

**4. Ophthalmoscopic examination -**

There were no treatment-related ophthalmological changes in any dose group throughout the study.

**E. Blood analyses****1. Haematology -**

There was an increase in the number of leukocytes in males at 20000 ppm but it reversed during the recovery period (Tab. 3). This change was considered toxicologically significant.

**2. Clinical Chemistry -**

At the end of the treatment period, activity of alkaline phosphatase (ALP) was elevated, and plasma triglyceride levels were decreased in males, while plasma cholesterol levels were increased in females, at 20000 ppm (Table 3). Activity of AST was decreased in males at 1250 ppm and above, and in females at 20000 ppm. Activity of GLDH was also decreased at 1250 ppm and above in males, in females at 5000 ppm and above. The decreases in these serum enzymes were not considered toxicologically significant as they normally go in the opposite direction if there is liver injury. In the microsomal enzyme assays, activity of N-demethylase was increased in females at 20000 ppm and that of O-demethylase increased in both sexes at 5000 ppm and above. Cytochrome P-450 content was elevated in males at 20000 ppm and in females beginning with 1250 ppm and above. These changes in liver microsomal enzymes in absence of histopathology were considered indicators of enzyme induction, rather than specific indicators of overt toxicity.

**F. Urinalysis -**

There were no treatment related alterations in urine parameters.

**G. Sacrifice and Pathology:****1. Organ weight -**

Notable organ weight changes are presented in Table 4. Significant increases were noted in absolute liver weights of females (14%), and relative liver weights of males (9-17%) and females (8-22%) at 5000 and above, and were reversible during the recovery period. The relative liver weight changes at 5000 ppm were not accompanied by any clinical biochemical or histological changes and therefore, were considered an adoptive response. At 20000 ppm however, the liver weight changes were considered by the reviewer to be toxicologically significant in view of the accompanying increase in alkaline phosphatase and decreased plasma glycerides in males, and increased plasma cholesterol in females. There was also increase in the absolute heart weight of females at 20000 ppm, but it was not accompanied by histopathological or biochemical changes, hence, considered incidental.

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Table 3. Haematology and clinical chemistry findings

Dose	0 ppm			1250 ppm		5000 ppm		20000 ppm		
Males										
Week	4	13	18 (re)	4	13	4	13	4	13	18 (re)
LEUCO [10 <sup>9</sup> /l]	9.7	9.1	10.0	11.0+	11.0	11.1*	9.8	12.2**	12.3**	9.5
AST [U/l]	38.7	39.1	34.0	33.4**	34.2	31.2**	30.1**	32.8**	29.8**	33.3
GLDH [U/l]	3.4	5.8	7.3	3.0	3.1**	2.8**	1.7**	2.7**	1.6**	6.5
ALP [U/l]	561	231	212	535	227	572	249	638*	254	213
CHOL [mmol/l]	2.11	2.41	2.76	2.27	2.49	2.28	2.66	1.79**	2.60	2.66
TRIGL [mmol/l]	1.99	1.61	1.98	1.56	1.87	1.69	1.67	1.23*	0.99**	1.82
Week		14	18 (re)		14		14		14	18 (re)
O-DEM [mU/g]		10.3	10.5		11.1		13.3**		14.6**	11.9*
P-450 [nmol/g]		36.8	38.4		38.2		42.0		49.2**	39.8
Females										
Week	4	13	18 (re)	4	13	4	13	4	13	18 (re)
AST [U/l]	37.2	39.8	38.7	36.2	38.0	34.0	40.9	33.6	33.3*	41.3
GLDH [U/l]	6.3	6.4	10.6	2.2	3.7	1.9*	1.8	1.5**	0.3**	15.3
CHOL [mmol/l]	2.09	2.39	2.39	2.23	2.46	2.22	2.67	2.33	3.24**	2.47
Week		14	18 (re)		14		14		14	18 (re)
N-DEM [mU/g]		72.7	75.4		71.3		83.0		88.2*	78.7
O-DEM [mU/g]		8.8	9.9		9.8		11.7**		16.7**	10.2
P-450 [nmol/g]		31.2	34.1		36.5*		40.4**		45.3**	30.7

\* U-test, 5 % significance level; \*\* U-test, 1 % significance level; re = recovery groups

Table 4. Organ weights (liver)

	0 ppm		1250 ppm	5000 ppm	20000 ppm	
	main group	recov. group	main group	main group	main group	recov. group
<b>Males</b>						
Absolute liver weights [g]	14.8	15.4	15.2	16.5	16.5	14.5
rel. liver weights [mg/100 g bw]	3392	3248	3493	3697*	3958**	3258
Absolute heart weights [g]	1.247	1.443	1.265	1.385	1.222	1.44
<b>Females</b>						
abs. liver weights [g]	8.015	7.543	7.918	8.535	9.105**	7.878
rel. liver weights [mg/100 g bw]	3393	2962	3322	3678*	4147**	3303
Absolute heart weights [g]	0.874	0.896	0.861	0.823	.788**	0.843

\*\*  $p \leq 0.01$ , \* =  $P \leq 0.05$ , recov. = recovery groups

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## 2. Gross pathology -

Necropsy indicated that 6 of 9 males at 20000 ppm had pale livers. There were no other treatment-related gross necropsy findings at any dose level.

## 3. Histo pathology -

There were no treatment related histological findings at any dose level.

# III. DISCUSSION

## A. Investigators' conclusions:

Taken together the results (from this study) may indicate an influence of treatment on liver function beginning at 5000 ppm. As most differences were small and had no related histological changes, the effects are not regarded as toxicologically significant, but an indication of weak inducing effect on microsomal enzymes with concomitant slight effect on lipid metabolism. Thus under conditions described, administration of SZX0722 was tolerated without toxic effects in doses up to and including 5000 ppm.

The NOAEL in both sexes was 5000 ppm, equal to 372.7 mg/kg bw/day (males), 561.4 mg/kg bw/day (females), based on reduced body weight gain, increased feed intake (only females), changed clinical chemistry parameters (including liver enzyme induction) and elevated absolute liver weights at 20000 ppm..

## B. Reviewer comments:

The increase in leucocyte counts in males at 20000 ppm which was reversed following withdrawal of treatment was considered toxicologically significant. Likewise, the liver weight changes observed at 20000 ppm in both sexes were considered to be toxicologically significant in light of the accompanying changes in alkaline phosphatase, and/or plasma cholesterol and plasma glycerides. The induction of the O-demethylase activity and cytochrome P-450 mono-oxygenase system (P-450) in liver tissue in both sexes were attributable to adoptive liver enzyme induction at that dose, and were not regarded as an adverse effect.

**The LOAEL in both sexes was 20000 ppm (1524.0 or 2585.9 mg/kg bw/d in males and females respectively), based on treatment related increased food intake with decrease in body weight gain and food efficiency, and increased plasma cholesterol levels in females, decrease in plasma triglycerides and increases in leukocyte counts, alkaline phosphatase levels and incidence of pale livers in males, as well as increased relative liver weights, in both sexes. The NOAEL was 5000 ppm; (372.7/561.4 mg/kg bw/day in males/females).**

IV. Study deficiencies: There were no major deficiencies that would affect the acceptability of this study.